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ABSTRACT

This study assessed, for the first time, the mycotoxicological risks from consumption of complementary foods by infants and young children in Nigeria. Molds belonging to *Aspergillus aculeatinus*, *A. flavus*, *A. luchuensis*, *A. tubingensis*, *A. welwitschiae* and *Geotrichum candidum* were recovered from the complementary foods. Twenty-eight major mycotoxins and derivatives, and another 109 microbial metabolites including chloramphenicol (a bacterial metabolite), were quantified in 137 food samples by LC-MS/MS. Aflatoxins and fumonisins co-contaminated 42% of the cereal- and nut-based food samples, at mean concentrations exceeding the EU limits of 0.1 and 200 μg/kg set for processed baby foods by 300 and six times, respectively. Milk contained mainly beauvericin, chloramphenicol and zearalenone. The trichothecenes, T-2 and HT-2 toxins, were quantified only in infant formula and at levels three times above the EU indicative level of 15 μg/kg for baby food. Chronic exposure estimate to carcinogenic aflatoxin was high causing low margin of exposure (MOE). Exposures to other mycotoxins either exceeded the established reference values by several fold or revealed low MOEs, pointing to important health risks in this highly vulnerable population. The observed mycotoxin mixtures may further increase risks of adverse health outcomes of exposure; this warrants urgent advocacy and regulatory interventions.

1. Introduction

Poor nutrition remains a major contributor to the numerous health problems faced by infants and young children (IYC) in resource-poor settings. Approximately one-third of children less than five years of age in developing countries suffer from nutrition-related health concerns (Onis and Branca, 2016; UNICEF, 2012). The period from birth to two years of age is the critical window for the promotion of optimal growth, health and development (WHO, 2012). However, nutrition during this age window in many parts of Africa is compromised by chronic and acute under-nutrition which impacts the survival, health and development of IYC (IARC, 2015). In particular, the presence of diverse mycotoxins in the daily diets of IYC can potentially affect child health, growth and development (Gong et al., 2002, 2003, 2004; IARC, 2015; Kamala et al., 2016; Kimanya et al., 2009, 2010, 2014; Turner, 2013; Turner et al., 2003, 2007).

Over 400 fungal metabolites are known, with more than 300 of them being addressed as mycotoxins (Zain, 2011). Mycotoxins, toxic secondary metabolites, are produced by some fungal species in crops and processed foods under certain favorable conditions of moisture, water activity, and temperature. A variety of mycotoxins including aflatoxins (AFs), fumonisins (FBs), citrinin (CIT), ochratoxin A (OTA),

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trichothecenes (deoxynivalenol (DON) and nivalenol (NIV)) and zearalenone (ZEN) have been reported to co-contaminate an array of agricultural produce (e.g. cereals, legumes, nuts, oilseeds and spices) and their products in Nigeria (Abdus-Salaam et al., 2015; Adetunji et al., 2014b; Ezekiel et al., 2012, 2016; Ogara et al., 2017; Okeke et al., 2015, 2018; Oyedele et al., 2017). Complementary foods are typically included in the diet for IYC when breast milk is no longer enough to meet the nutritional needs. Cereals and nuts together with milk and their products are the major components of complementary foods for IYC in Nigeria; as such, young children may be exposed to diverse mycotoxins through consumption of contaminated diets at the weaning stage. Unlike the adults, young children are considered as the most vulnerable population in terms of mycotoxin exposure. This is mainly due to their young age, high intake of food and water per kilogram body weight, fairly restricted diet, rapid rate of metabolism and growth, and a lower detoxification capacity (Alvito et al., 2010; EFSA, 2007; Lombard, 2014; WHO, 2006).

Mycotoxin contamination of complementary foods fed to infants in the form of milk, infant formula, cereal- and nut-based foods have been reported in other African countries (Gong et al., 2003, 2004; Kamala et al., 2016; Kimanya et al., 2010). However, there is a paucity of report on mycotoxin contamination of complementary foods from Nigeria. There has also been no effort to characterize the health risks associated with chronic dietary mycotoxin exposure from complementary foods in IYC. In the literature, however, there are reports on aflatoxin M_1 contamination of breast milk from lactating mothers and suggested risk of exposure for IYC (Adejumo et al., 2013), mycotoxin exposures based on urinary biomarker measurements of only 19 children (< 8 years) who consumed general home diets made from mainly maize (Ezekiel et al., 2014), and risk assessment estimations for IYC based on unprocessed raw maize and peanut in storage (Adetunji et al., 2017; Oyedele et al., 2017). Assessing complementary food-based mycotoxin exposure in IYC in Nigeria is, therefore, necessary for an in-depth understanding of the pattern and extent of dietary exposure to the toxins, as well as for assessing the possible chronic health risks this population may face through the daily consumption of mycotoxin-contaminated foods. In addition, a risk assessment is highly necessary in Nigeria, where mycotoxin regulations and monitoring of foods for IYC seem inadequate. Risk assessment provides reliable scientific evidence on which legislation for food regulation can be enacted and other risk management actions established in order to protect the health of IYC.

Presently, only AFs are regulated in Nigeria and in most foods, including baby foods, the limit is set at 4 μg/kg. By contrast, the regulatory maximum levels established by the European Union (EU) are: 0.10 μg/kg for aflatoxin B_1 (AFB_1) in baby foods and processed cereal-based foods for IYC; 0.025 μg/kg for AFM_1 in infant formula and follow-on formulae including infant milk and follow-on milk; 200 μg/kg for DON in processed cereal-based foods for IYC; 200 μg/kg for FBs (sum of FB_1 and FB_2) in processed maize-based foods and baby foods for IYC; 0.50 μg/kg for OTA in processed cereal-based foods and baby foods for IYC; 20 μg/kg for ZEN in processed cereal-based and processed maize-based foods and baby foods for IYC; and 15 μg/kg (indicative level, not a safety level) for the sum of T-2 and HT-2 in cereal-based foods for IYC (EC, 2006, 2013).

This study, therefore, aimed to assess the mycotoxicological risks associated with the consumption of complementary foods by IYC in Nigeria. To achieve this, complementary foods were analyzed for fungal species and a broad spectrum of microbial metabolites including fungal toxins and bacterial metabolites. Furthermore, the levels of exposure and risks that may result from daily consumption of complementary foods by IYC were estimated. It is envisaged that this study would provide expository and reliable food contamination and potential health risks data, and thus serve as a reference point to support mycotoxin monitoring and establishment of stiffer regulations for IYC foods in Nigeria.

2. Materials and methods

2.1. Description of study and food sampling

Complementary food samples (n = 137) that are routinely fed to IYC by their mothers and caregivers in Lagos and Ogun states, Nigeria, were collected in a longitudinal pattern. The longitudinal pattern consisted of two sampling rounds (hot dry season: 54 samples; warm wet season: 83 samples). The samples of the hot dry season were collected in January 2017, while those of the warm wet season were collected in June 2017. The distributions of samples were based on food preference/utilization and the food types included family cereal (maize-based pudding) (n = 26), peanut butter (n = 5), *ogi* (fermented maize pudding) (n = 23), *Tom bran* (whole meal from mixed grains (including maize and peanut) (n = 30), powdered milk (n = 36), and infant formula (milk- and cereal (maize, oats, rice or wheat)- based) (n = 17). The distributions (number of samples) of food types collected during the seasons were family cereal (hot dry season = 11; warm wet season = 15), peanut butter (hot dry season = 2; warm wet season = 3), *ogi* (hot dry season = 6; warm wet season = 17), *Tom bran* (hot dry season = 14; warm wet season = 16), powdered milk (hot dry season = 15; warm wet season = 21), and infant formula (hot dry season = 5; warm wet season = 12).

Food samples were collected at the point of feeding, either at the households or caregiver institution, after appropriate consent of mothers and caregivers were obtained and documented. Participation in the study was voluntary, only children aged 6–24 months without present ill-health as observed by mother/caregiver were included in the study approved by the Babcock University Health Research Ethics Committee (authorization number: BUHREC524/17). Data on socio-economic status of households, anthropometric data, food consumption pattern, dietary preference, and health status of 110 infants were collected by using a well-structured food frequency questionnaire (Ezekiel et al., 2014) administered to mothers and caregivers (full details in another paper, *in preparation*).

Food samples collected in this study were in the undiluted or uncooked form. For the sampling of commercial complementary foods (family cereal, peanut butter, powdered milk and infant formula), samples were taken from households with at least 400–500 g of specific food item; this was considered as the bulk sample. For the household-formulated complementary foods (*Tom bran* and *ogi*), bulk sample (500 g) of each food was collected in the first instance from at least five parts of the large food containers. Food samples weighing 20 g were then subsampled randomly and aseptically from each bulk sample and divided into two batches: batch A (10 g) for mycological analysis, and batch B (10 g) for mycotoxin analysis. Batch A samples were analyzed within 24 h of collection, while batch B samples were frozen (−20 °C) until analyzed.

2.2. Mycological analysis of food samples

2.2.1. Isolation and enumeration of fungi

Molds in the complementary food samples were isolated and enumerated on Dichloran-Glycerol (DG18) agar after dilution plating (Samson et al., 1995) was performed on the samples. The inoculated plates were incubated in the dark at 30 °C for 3 days and fungal colony counts were reported as colony forming units per gram of food sample (CFU/g).

2.2.2. Morphological assessment of fungal isolates

Colonies resembling *Aspergillus* (characteristic black and yellow-green colonies of *Aspergillus* section *Nigri* and *Aspergillus* section *Flavi*, respectively) and those of other fungi were carefully transferred with sterile toothpicks to a set of three mycological media (DG18, yeast extract sucrose (YES) agar, and malt yeast supplemented with 40% sucrose (M40Y) agar) for the assessment of colonial features after 7

days of growth at 30 °C (Samson et al., 1995).

2.2.3. Molecular identification and phylogenetic analysis of fungal isolates

Genomic DNA was extracted from the pure fungal isolates by using ZR Fungal/Bacterial DNA Miniprep kit (Zymo Research, California, USA) as previously described by Oladipo et al. (2016). Isolates were initially identified based on the sequences of the full internally transcribed spacer 1 (ITS1)-5.8S-internally transcribed spacer 2 (ITS2) region (hereafter referred to as only ITS) by using primers ITS1 (5′-TCC GTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATA TGC-3′) (White et al., 1990). Thereafter, for species-level resolution of closely-related filamentous (*Aspergillus*) species, the beta-tubulin (*benA*) and calmodulin (*caM*) genes were amplified and sequenced using primer sets ben2f (5-TCCAGACTGGTCAGTGTGTAA-3)/bt2b (5′-ACCC TCAGTGTAGTGACCCTTGGC-3′) (Glass and Donaldson, 1995; Hubka and Kolarik, 2012) and CMD5 (5′—CCGAGTACAAGGAGGCCTTC-3′)/ CMD6 (5′- CCGATAGAGGTCATAACGTGG-3′) (Hong et al., 2005), respectively (see supplementary Table A.1. for more information). Taxonomic assignments were conducted by aligning sequences against public sequences in the UNITE database (https://unite.ut.ee/), and GenBank. Furthermore, curated sequences of the ITS, partial *benA* and *caM* genes (for *Aspergillus* species only) of closely related type strains in the GenBank were selected for phylogenetic analysis (see supplementary File A.1 and Table A.2. for more information).

2.3. Multi-mycotoxin analysis of food samples

The dilute and shoot LC-MS/MS method described by Malachová et al. (2014) was employed to quantify mycotoxins and other microbial metabolites in the complementary food samples.

2.3.1. Chemicals

Methanol (LC gradient grade) and glacial acetic acid (p.a) were purchased from Merck (Darmstadt, Germany), acetonitrile (LC gradient grade) from VWR (Leuven, Belgium), and ammonium acetate (MS grade) from Sigma-Aldrich (Vienna, Austria). Mycotoxin standards were obtained as donations from various research groups or purchased from various commercial sources. Water was purified successively by reverse osmosis with an Elga Purelab ultra analytic system from Veolia Water (Bucks, UK).

2.3.2. Extraction of metabolites and estimation of matrix effects

Representative food sample (5 g) was homogenized with 20 ml of extraction solvent (acetonitrile/water/acetic acid 79:20:1, v/v/v) in a 50 ml polypropylene tube (Sarstedt, Nümbrecht, Germany). All samples were extracted for 90 min on a GFL 3017 rotary shaker (GFL, Burgwedel, Germany) and diluted with the same volume of the extraction solvent. Since diluted extracts were sufficiently sedimented by gravity, diluted extracts were directly injected into the LC-MS/MS instrument (Sulyok et al., 2007) without a need for a centrifugation step. Apparent recoveries of the analytes were determined by spiking 0.25 g of five different samples. The spiked samples were stored overnight at ambient temperature to allow evaporation of the solvent and to establish equilibrium between the analytes and samples. The extraction (in 1 ml of solvent), dilution and analysis were as described earlier. The accuracy of the method was verified by participation in inter-laboratory comparison studies organized by BIPEA (Gennevilliers, France). Currently, 94% of the > 850 results submitted for different types of grains, nuts, dried fruits, spices, baby food, milk powder and animal feed were in the satisfactory range (z-score between −2 and 2).

2.3.3. LC-MS/MS parameters

LC-MS/MS screening of the microbial metabolites was performed with a QTrap 5500 LC-MS/MS System (Applied Biosystem, Foster City, CA, USA) equipped with TurboIonSpray electrospray ionisation (ESI) source and a 1290 Series HPLC System (Agilent, Waldbronn, Germany). Chromatographic separation was performed at 25 °C on a Gemini® C18-column, 150 × 4.6 mm i.d., 5 µm particle size, equipped with a C18 4 × 3 mm i.d. security guard cartridge (Phenomenex, Torrance, CA, USA). The chromatographic method, chromatographic and mass spectrometric parameters are as described by Malachová et al. (2014). ESI-MS/MS was performed in the time-scheduled multiple reaction monitoring (MRM) mode both in positive and negative polarities in two separate chromatographic runs per sample by scanning two fragmentation reactions per analyte. The MRM detection window of each analyte was set to its expected retention time ± 27 s and ± 48 s in the positive and the negative modes, respectively. Confirmation of positive analyte identification was obtained by the acquisition of two MRMs per analyte (with the exception of moniliformin (MON), which exhibited only one fragment ion). This yielded 4.0 identification points according to European Commission decision 2002/657 (EC, 2002). In addition, the LC retention time and the intensity ratio of the two MRM transitions agreed with the related values of an authentic standard within 0.1 min and 30% respectively.

2.4. Mycotoxin exposure and risk assessment of IYC

2.4.1. Chronic exposure assessment by the deterministic approach

Due to the chronic nature of the adverse effects induced by the tested mycotoxins, a chronic dietary exposure to these mycotoxins was assessed. To assess chronic mycotoxin exposures in the IYC who consume the complementary foods, a deterministic approach was used and a single point estimate of mycotoxins performed. Exposure assessment methods are appropriate to any food-borne chemical and probable daily intake (PDI) is a simple way to assess exposure to chemicals in foods (Codex Alimentarius, 1989; IPCS, 2009). PDI has been used in other studies (Fernandes et al., 2011; Kimanya et al., 2010; Marin et al., 2011; Törnkvist et al., 2011). To estimate the chronic PDI of each mycotoxin for the IYC, firstly, the daily consumption of the complementary foods (g/day) was derived from the data provided in the questionnaires for all 110 subjects included in the study. The weighted meal eaten at a single eating occasion was multiplied by the number of eating occasions per day to derive the daily food consumption per infant. The food consumption at an individual infant level was the estimate of the mother or caregiver. The daily consumption per child was based on the most frequently consumed single complementary food item, although consumption of other complementary food items was also reported. The food consumption data were collected on a single day. Then, the daily amount of the consumed food by the infant (g/day) was multiplied with the mycotoxin concentration (ng/g) in the food consumed by the infant to obtain the mycotoxin intake. The mycotoxin exposure of the individual infant (ng/kg bw per day) was then calculated by dividing the mycotoxin intake with the body weight (kg) of that infant.

The chronic dietary exposures of IYC to AFB_1, total AFs, fumonisin B_1 (FB_1), $FB_1 + FB_2$, $FB_1 + FB_2 + FB_3$, OTA and CIT were estimated as these are the major mycotoxins of significance in relation to public health. Of the emerging mycotoxins (Jestoi, 2008), chronic exposures to BEA and MON were assessed.

Handling data below the limit of detection (LOD) (so-called left-censored data) is important for exposure assessment and consequently for risk assessment. Mycotoxin contamination in the complementary food samples was evaluated and values below the limit of detection (LOD) were considered in accordance with the IPCS/GEMS (1995) criteria adopted to estimate mycotoxin exposure when concentrations less than the LOD are observed. The sample sets for different food groups analyzed varied from 17 samples of infant formula to 36 samples of milk. For peanut butter, only five samples were analyzed. The proportion of samples above LOD was high for most of the mycotoxins and the proportions ranged from 60% to 100% in many of the food groups. Of the major mycotoxins, only OTA had a high amount (80–100%) of left-censored data in all food groups, while AFB_2 and AFG_2 were only high in some food groups. High proportion of positive results was also

Table 1
Occurrence levels of 29 mycotoxins and one bacterial metabolite in 84 cereal- and nut-based complementary foods from Nigeria.

Mycotoxins and derivatives	Family cereal (n[a] = 26; N[b] = 16)				Peanut butter (n[a] = 5; N[b] = 6)				*Ogi* (n[a] = 23; N[b] = 19)				*Tom bran* (n[a] = 30; N[b] = 27)			
	%p[c]	Concentration (μg/kg)			%p[c]	Concentration (μg/kg)			%p[c]	Concentration (μg/kg)			%p[c]	Concentration (μg/kg)		
		Range	Median	Mean ± SD[d]		Range	Median	Mean ± SD[d]		Range	Median	Mean ± SD[d]		Range	Median	Mean ± SD[d]
3-Nitropropionic Acid	61.5	1.6–18.5	7.9	8.5 ± 4.2	20.0	5.4	5.4	5.4 ± 0.0	4.3	3.7	3.7	3.7 ± 0.0	86.7	5.7–993	19.9	79.2 ± 198
Aflatoxicol	0.0	< LOD	–	–	0.0	< LOD	–	–	0.0	< LOD	–	–	20.0	1.4–7.8	3.7	4.4 ± 2.7
Aflatoxin B_1	92.3	0.4–9.3	1.6	2.4 ± 2.4	80.0	5.5–11.6	6.9	7.7 ± 2.8	52.2	0.4–42.8	1.0	4.8 ± 12.0	80.0	1.3–474	27.2	83.4 ± 125
Aflatoxin B_2	19.2	0.5–0.8	0.7	0.7 ± 0.1	80.0	0.6–2.0	1.3	1.3 ± 0.6	4.3	2.4	2.4	2.4 ± 0.0	56.7	0.6–81.8	4.6	12.7 ± 19.6
Aflatoxin G_1	92.3	0.4–2.5	0.9	1.0 ± 0.5	0.0	< LOD	–	–	34.8	0.4–1.6	0.8	0.8 ± 0.4	53.3	0.4–237	3.6	22.1 ± 58.5
Aflatoxin G_2	0.0	< LOD	–	–	0.0	< LOD	–	–	0.0	< LOD	–	–	13.3	1.4–20.7	3.4	7.2 ± 9.1
Total aflatoxins[e]	100	0.4–11.1	2.3	3.3 ± 2.9	80.0	6.5–13.6	8.0	9.0 ± 3.3	52.2	0.4–46.8	1.7	5.5 ± 13.0	83.3	0.5–590	31.2	104 ± 166
Aflatoxin M_1	0.0	< LOD	–	–	0.0	< LOD	–	–	4.3	0.9	0.9	0.9 ± 0.0	46.7	0.9–24.4	3.2	5.4 ± 6.3
Aflatoxin P_1	0.0	< LOD	–	–	0.0	< LOD	–	–	0.0	< LOD	–	–	3.3	14.6	14.6	14.6 ± 0.0
Alternariol	15.4	0.4–0.9	0.5	0.6 ± 0.2	0.0	< LOD	–	–	4.3	0.4	0.4	0.4 ± 0.0	30.0	1.2–7.2	1.4	2.1 ± 1.9
Beauvericin	69.2	0.1–0.6	0.2	0.2 ± 0.1	80.0	0.6–4.1	1.1	1.7 ± 1.6	82.6	0.1–5.3	0.9	1.3 ± 1.4	96.7	0.1–69	0.5	4.4 ± 13.1
Chloramphenicol	30.8	0.2–105	0.6	13.7 ± 36.7	20.0	3.3	3.3	3.3 ± 0.0	17.4	0.1–1.7	0.9	0.9 ± 0.8	50.0	0.2–42.5	1.1	10.9 ± 15.9
Citrinin	88.5	1.2–151	25.1	32.6 ± 39.8	0.0	< LOD	–	–	60.9	0.8–159	7.0	20.0 ± 40.8	73.3	1.7–1173	13.4	160 ± 313.6
Deoxynivalenol	0.0	< LOD	–	–	0.0	< LOD	–	–	0.0	< LOD	–	–	6.7	30.8–31.6	31.2	31.2 ± 0.6
Dihydrocitrinone	7.7	2.2–3.4	2.8	2.8 ± 0.8	0.0	< LOD	–	–	4.3	3.9	3.9	3.9 ± 0.0	53.3	2.4–210	13.3	31.9 ± 51.1
Fumonisin A_1	0.0	< LOD	–	–	0.0	< LOD	–	–	43.5	1.2–11.3	3.4	4.4 ± 3.6	16.7	2.2–4.3	3.0	3.1 ± 0.8
Fumonisin A_2	65.4	2.4–42.6	16.2	16.7 ± 10.3	0.0	< LOD	–	–	43.5	5.3–42.3	12.7	16.1 ± 12.2	26.7	3.2–26.4	13.1	13.6 ± 9.1
Fumonisin B_1	100	43–836	176.4	245 ± 195	0.0	< LOD	–	–	82.6	16.1–562	104.1	163.4 ± 169	90.0	11–974	63.1	143.5 ± 200
Fumonisin B_2	100	19.3–267	60.4	84.5 ± 62.7	0.0	< LOD	–	–	87.0	15.0–403	86.3	112.5 ± 102	86.7	7.1–318	22.7	48.9 ± 64.9
Fumonisin B_3	92.3	12.4–152	48.2	48.3 ± 35.9	0.0	< LOD	–	–	60.9	7.4–100	30.7	41.5 ± 27.6	50.0	12.7–143	25.8	38.8 ± 33.5
Total fumonisins[f]	100	62.3–1103	236.7	329 ± 258	0.0	< LOD	–	–	87.0	32.4–837	190.3	268 ± 263	96.7	7.8–1293	82.0	178 ± 259
Total fumonisins[g]	100	62.3–1255	265.4	374 ± 294	0.0	< LOD	–	–	87.0	32.4–910	212.9	297 ± 292	96.7	7.8–1436	93.1	197.6 ± 289
Fumonisin B_4	96.2	7.3–109	28.0	33.8 ± 25.7	0.0	< LOD	–	–	78.3	3.8–222	45.9	55.1 ± 49.5	60.0	3.7–105	13.7	22.9 ± 25.0
Hydrolysed FB_1	0.0	< LOD	–	–	0.0	< LOD	–	–	0.0	< LOD	–	–	10.0	2.1–8.1	6.9	5.7 ± 3.1
Moniliformin	92.3	1.7–34.8	7.8	9.8 ± 7.4	60.0	2.2–3.5	2.7	2.8 ± 0.6	17.4	2.4–32.3	5.1	11.2 ± 14.2	96.7	5.1–3450	28.0	165 ± 635
Nivalenol	0.0	< LOD	–	–	0.0	< LOD	–	–	0.0	< LOD	–	–	10	11.4–23.8	14.1	16.4 ± 6.6
Ochratoxin A	7.7	0.5–0.5	0.5	0.5 ± 0.0	0.0	< LOD	–	–	8.7	0.7–1.8	1.2	1.2 ± 0.8	26.7	0.5–26.4	3.4	6.8 ± 8.9
Ochratoxin B	0.0	< LOD	–	–	0.0	< LOD	–	–	0.0	< LOD	–	–	10	3.5–113	16.0	44 ± 59.8
Tenuazonic acid	0.0	< LOD	–	–	0.0	< LOD	–	–	0.0	< LOD	–	–	13.3	41.4–292	61.1	114 ± 120
Zearalenone (ZEN)	0.0	< LOD	–	–	0.0	< LOD	–	–	8.7	0.4–2.7	1.6	1.6 ± 1.6	13.3	0.6–10.3	4.0	4.7 ± 4.1

[a] Number of samples analyzed.
[b] Number of mycotoxins and bacterial metabolite detected by LC-MS/MS.
[c] Percent positive samples.
[d] Mean and standard deviation from mean of toxin levels found in samples.
[e] Summation of aflatoxin B_1, aflatoxin B_2, aflatoxin G_1 and aflatoxin G_2 levels.
[f] Summation of fumonisin B_1 and fumonisin B_2 levels.
[g] Summation of fumonisin B_1, fumonisin B_2 and fumonisin B_3 levels.

observed for the emerging toxins in most of the food groups. When the proportion of mycotoxin results with < LOD was below 60% for the specific food items, and the food item consumed by a child reported a left-censored result, the exposure of that child was calculated by applying LOD/2 (= middle bound) to substitute the left-censored result. This was considered to give an appropriate exposure estimate because the proportion of left-censored data was low and the LOD for the mycotoxin was the same (or it was close in case of different forms of AFs) (IPCS, 2009). For the OTA data and for the other data sets, where the proportion of the left-censored data was greater than 60%, the substitution method, as recommended by IPCS (2009) and further by EFSA (2010) to use the lower bound (LB) and upper bound (UB) approach for chemicals which are likely present in the food (e.g. mycotoxins), was applied. To calculate the LB and UB exposure estimates, the mycotoxin concentrations < LOD were substituted with a value of 0 (i.e. minimum possible value) and with a value of LOD (i.e. maximum possible value), respectively. The food items which had mycotoxin levels < LOD for all samples tested (see Table 1) were not considered in the exposure assessment. Thus, for example, for the dietary exposure to FBs, peanut butter did not contribute nor did milk. A mean weekly exposure at the LB and UB was calculated for OTA assuming a consumption of the same food items with the same daily amount over seven days.

2.4.2. Risk characterization of the mycotoxins in complementary foods

To characterize the risk from the exposure to mycotoxins, the estimated exposures (PDIs) were compared with the defined reference point for aflatoxin, and with established health-based guidance values (HBGVs) or other reference points for other mycotoxins (IPCS, 2009). The margin of exposure (MOE) approach recommended by EFSA (2005, 2007) and Benford et al. (2010) was applied to estimate the risk from exposure to AFB_1 due to its genotoxic and carcinogenic nature. Consequently, it is assumed that any exposure, even at low doses, may pose a potential health risk (EFSA, 2005). The MOE was calculated by dividing the benchmark dose lower confidence limit of 10% extra risk ($BMDL_{10}$) of 170 ng/kg bw per day calculated for AFB_1 from rodent data (EFSA, 2007) by the estimated exposure (PDI) for AFB_1. The exposure (PDI) was also calculated for the sum of AFs (AFB_1, AFB_2, AFG_1 and AFG_2, i.e. total AFs). However, the $BMDL_{10}$ is for AFB_1 only. Therefore, in order to be conservative noting that AFB_1 constituted the major part of the total AFs in the analyzed samples, the same assumption as made by EFSA (2007) on similar toxicological potency of the total AFs to AFB_1 was taken. Thus, the $BMDL_{10}$ of 170 ng/kg bw per day was used as a reference value to also assess the risk posed by exposure to total AFs in the daily diets of the IYC.

To characterize the risk from the exposure to FBs and OTA, a group tolerable daily intake (TDI) of 2 μg/kg bw per day for FB_1, FB_2 and FB_3 recommended by JECFA (2011) and tolerable weekly intake (TWI) of 0.1 μg/kg bw per week recommended by JECFA (2008), respectively, were applied. For CIT, as no HBGV (e.g. TDI or TWI) has been established due to its potential genotoxic and carcinogenic properties and substantial uncertainties in the available toxicity data (EFSA, 2012), the approach of EFSA to characterize the risk for nephrotoxicity by using the level of no concern for nephrotoxicity of 0.2 μg CIT/kg bw per day was considered appropriate. Therefore, MOE was calculated with this value. At this level of exposure, however, a concern for genotoxicity and carcinogenicity remains as concluded by EFSA (2012). Due to the high uncertainties in the toxicity data, EFSA used a MOE approach for the risk characterization of BEA and MON (EFSA, 2014, 2018a). Therefore, this approach was applied. In line with EFSA, the risk assessments presented in this paper are indicative. To be prudent, the lowest dose of 90 μg BEA/kg bw per day identified by EFSA (2014) was selected for a reference point to calculate the MOE for BEA. The $BMDL_{05}$ of 200 μg/kg bw per day for MON derived by EFSA (2018b) was used to calculate the MOE for MON.

2.5. Statistical analysis

Data for food consumption and mycotoxin occurrences in the complementary foods were analyzed using SPSS Statistics package version 20.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics was performed for the distribution and levels of mycotoxins in the different types of complementary foods. Mean mycotoxin levels in the complementary foods collected across the two sampling seasons (hot dry and warm wet) were compared by analysis of variance (ANOVA) and the unpaired student t-test (two-sided). Where means were significant ($p < 0.05$), mean separation (post hoc test) was performed by the Duncan's Multiple Range test (DMRT) at 95% confidence level. Data for mycotoxin variations in foods by sampling season were represented in clustered boxplots.

3. Results

3.1. Demographic and food consumption data for the infants and young children

The study consisted of 60 male and 50 female subjects with a mean age of 15 months (range: 6–24 months). The mean age (± SD) of infants for the introduction of complementary foods was 18.3 ± 1.6 weeks. About 55% of the IYC were fed the complementary foods 1–3 times daily while the remaining proportion of the IYC consumed the foods 4–6 times daily. For all subjects, the complementary foods constituted at least 50% of their full diet per day due to consumption of additional food commodities (e.g. breast milk for subjects below one year of age, and cereal- and tuber-based foods for subjects above 12 months but less than 24 months). The average estimated weight (± SD) of the complementary food meals consumed by the subjects per day was 608 ± 179 g (range: 150–950 g). The mean (± SD) body weight and body mass index of the children were 9.8 ± 2.2 kg and 17.6 ± 3.4 kg/m^2, respectively.

3.2. Fungal species associated with complementary foods

The mean CFU/g of molds found in the complementary foods ranged from 200 to 1900 CFU/g (data not shown). Ninety-five fungal isolates were recovered from all samples and identified as *Aspergillus* (81.1%) and *Geotrichum candidum* (18.9%) (Fig. 1). Of the isolates belonging to *Aspergillus*, five phylotypes (Fig. 1a) were identified (frequency of occurrence in all food samples in parenthesis): *Aspergillus flavus* (33.7%), *A. welwitschiae* (28.4%), *A. tubingensis* (13.7%), *A. aculeatinus* (3.2%) and *A. luchuensis* (2.1%) (Fig. 2). Sequences of isolates are available in the GenBank under the accession numbers MH035980-MH035998 (ITS), MH063937-MH063951 (*benA*) and MH063952-MH063966 (*caM*). About 67% of all fungal isolates were obtained from *Tom bran* while only 2% was obtained from peanut butter. No fungal propagule was recovered from milk and infant formula.

3.3. Occurrence and distribution of microbial metabolites in complementary foods

3.3.1. Overview of microbial metabolites in complementary foods

A total of 137 metabolites (including 116 fungal-, 3 bacterial-, 1 plant-, and 17 unspecific metabolites) were quantified in the complementary food samples and the method performance for the metabolites are shown in Table A.3. Among these metabolites are those produced mainly by *Penicillium*, *Aspergillus* (including aflatoxin/sterigmatocystin pathway precursors), *Fusarium, Alternaria*, *Claviceps*, and other fungi. With respect to metabolite prevalence in the food samples, the *Aspergillus* metabolites were dominant (>40%) (Tables 1 and 2, Table A.4).

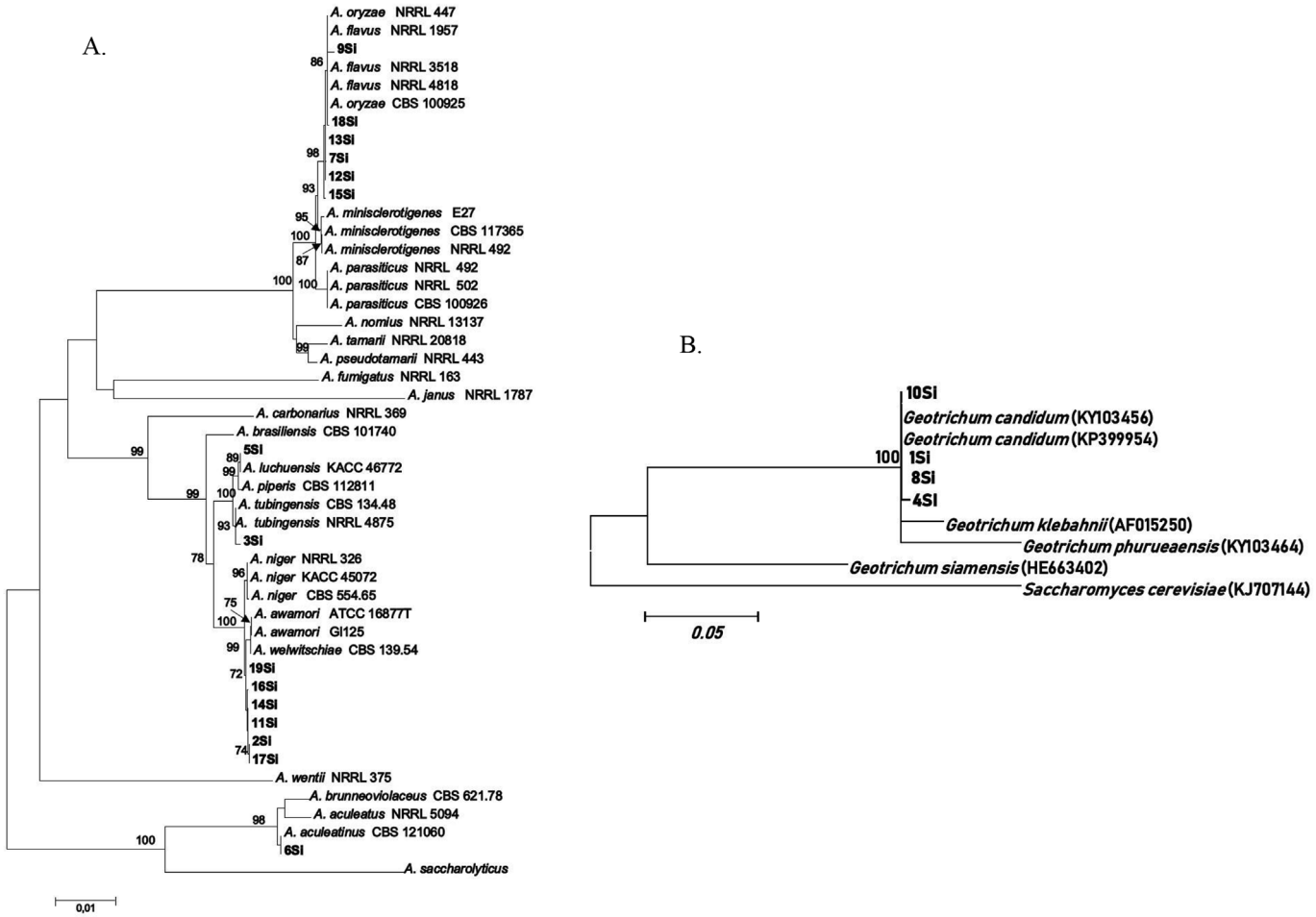

Fig. 1. Phylogenetic tree for *Aspergillus* and *Geotrichum* from complementary foods: Tree support was based on 1000 bootstrap replications. Only bootstrap values greater than 70% are shown. Isolates obtained in this study are in bold letters. **A.** The maximum-likelihood tree with the highest log-likelihood score based on concatenated dataset of ITS, partial *benA* and *caM* gene sequences of *Aspergillus* species. **B.** Maximum-likelihood tree with the highest log-likelihood score based on ITS sequences. Tree was rooted with *S. cerevisiae*.

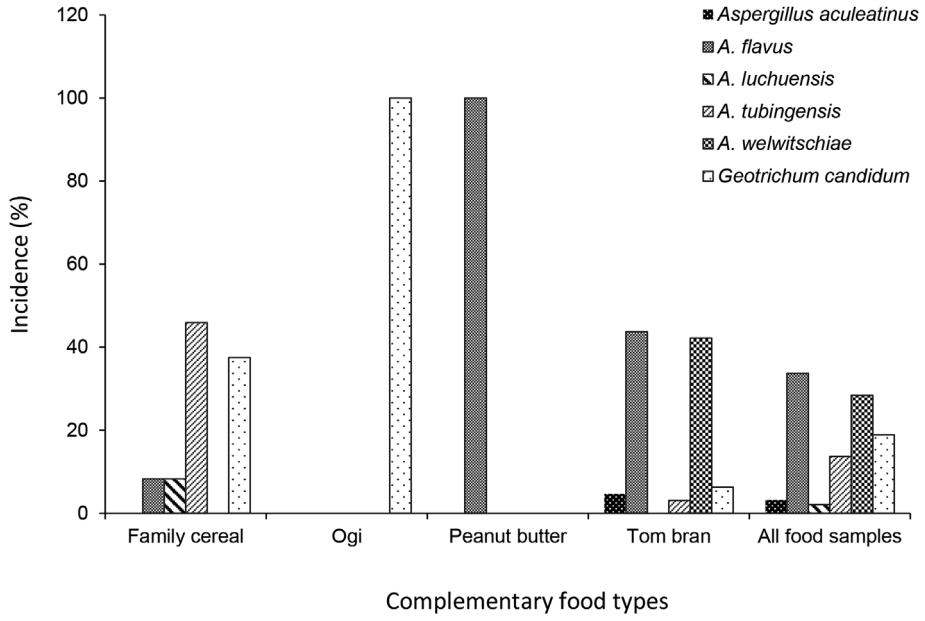

Fig. 2. Distribution of 95 fungal isolates in complementary foods in Nigeria. No fungal propagule was recovered from milk and infant formula.

Table 2
Distribution of 13 mycotoxins and one bacterial metabolite in 53 milk and infant formula from Nigeria.

Metabolites	Milk (n[a] = 36; N[b] = 3)				Infant formula (n[a] = 17; N[b] = 14)			
	% positive samples	Concentration (μg/kg)			% positive samples	Concentration (μg/kg)		
		Range	Median	Mean ± SD[c]		Range	Median	Mean ± SD[c]
3-Nitropropionic acid	0.0	< LOD	–	–	11.8	19.6–22.5	21.1	21.1 ± 2.0
Aflatoxin B_1	0.0	< LOD	–	–	5.9	4.2	4.2	4.2 ± 0.0
Aflatoxin B_2	0.0	< LOD	–	–	5.9	0.5	0.5	0.5 ± 0.0
Total aflatoxins[d]	0.0	< LOD	–	–	5.9	4.6	4.6	4.6 ± 0.0
Alternariol	0.0	< LOD	–	–	5.9	0.7	0.7	0.7 ± 0.0
Beauvericin	27.8	0.04–0.4	0.2	0.2 ± 0.1	17.6	0.1–13.4	0.5	4.7 ± 7.5
Chloramphenicol	38.9	0.1–63.8	0.6	8.5 ± 17.4	23.5	0.5–14.3	0.9	4.1 ± 6.7
Citrinin	0.0	< LOD	–	–	5.9	3.6	3.6	3.6 ± 0.0
Deoxynivalenol	0.0	< LOD	–	–	11.8	27.2–36	31.6	31.6 ± 6.3
HT-2 toxin	0.0	< LOD	–	–	5.9	18.8	18.8	18.8 ± 0.0
Moniliformin	0.0	< LOD	–	–	11.8	10.0–16.0	13.0	13 ± 4.2
Nivalenol	0.0	< LOD	–	–	11.8	18.9–22.0	20.5	20.5 ± 2.2
T-2 toxin	0.0	< LOD	–	–	11.8	0.8–119	59.6	59.6 ± 83.2
Zearalenone	2.8	0.6	0.6	0.6 ± 0.0	23.5	0.4–5.4	3.6	3.2 ± 2.1

[a] Number of samples analyzed.
[b] Number of mycotoxins and bacterial metabolite detected by LC-MS/MS.
[c] Average and standard deviation from mean of toxin levels found in samples.
[d] Summation of aflatoxin B_1 and aflatoxin B_2 levels.

3.3.2. Mycotoxin occurrences and levels in complementary foods

Twenty-eight major mycotoxins and their derivatives were quantified in the complementary food samples (Tables 1 and 2). Table 1 presents the distribution and occurrence levels of 29 mycotoxins (including the sum of AFB_1, AFB_2, AFG_1 and AFG_2; sum of FB_1, FB_2 and FB_3; and sum of FB_1 and FB_2) and one major occurring bacterial metabolite (chloramphenicol) in 84 cereal- and nut-based complementary food samples. Similarly, Table 2 shows the distribution of 13 mycotoxins and the bacterial metabolite (chloramphenicol) in 53 milk and infant formula samples. *Tom bran*, *ogi*, family cereal, infant formula, peanut butter and milk contained 27, 19, 16, 13, 6 and 3 different mycotoxins, respectively.

Aflatoxin B_1 contaminated 92.3% of family cereal, 80% of peanut butter, 80% of *Tom bran*, 52.2% of *ogi* and 5.9% of infant formula samples at respective mean concentrations of 2.4, 7.7, 83.4, 4.8 and 4.2 μg/kg. Total AFs (AFtot: sum of AFB_1, B_2, G_1 and G_2) occurred in 100% of family cereal (mean: 3.3 μg/kg), 83.3% of *Tom bran* (mean: 104 μg/kg), 80% of peanut butter (mean: 9 μg/kg), 52.2% of *ogi* (mean: 5.5 μg/kg) and 5.9% of infant formula (mean: 4.6 μg/kg) samples (Tables 1 and 2). Other AF types quantified were AFM_1 in 4.3% of *ogi* (0.9 μg/kg) and 47% of *Tom bran* (mean: 5.4 μg/kg), and AFP_1 in one sample of *Tom bran* (14.6 μg/kg) (Table 1). FBs contaminated only the cereal-based complementary foods, with FB_1 occurring in 100% of family cereal (mean: 245 μg/kg), 90% of *Tom bran* (mean: 143.5 μg/kg) and 82.6% of *ogi* (mean: 163.4 μg/kg) samples (Table 1). The mean concentration of total FBs (sum of FB_1, FB_2 and FB_3) was higher in family cereal samples (374 μg/kg) than in *ogi* (297 μg/kg) and *Tom bran* (198 μg/kg). FBs of the A series were quantified in the foods where the B series were also found. Ochratoxin A was quantified in family cereal (7.7%), *ogi* (8.7%) and *Tom bran* (26.7%) samples while ZEN occurred in only *ogi* (8.7%) and *Tom bran* (13.3%) samples (Table 1). HT-2 and T-2 were detected only in 5.9% and 11.8% of infant formula samples at the mean concentrations of 18.8 μg/kg and 59.6 μg/kg, respectively (Table 2). Deoxynivalenol occurred in 6.7% and 11.8% of *Tom bran* and infant formula samples, respectively, with mean concentrations of 31 μg/kg in each food type.

Citrinin and its metabolite, dihydrocitrinone, were found in at least 61% and 4%, respectively, of the cereal-based food samples (family cereal, *ogi* and *Tom bran*) although as many as 53% of *Tom bran* samples contained dihydrocitrinone (Table 1). Only one sample of infant formula with milk and maize as constituents, however, contained CIT (Table 2). Other prevalent mycotoxins were the emerging *Fusarium* mycotoxins, BEAU and MON, which contaminated as much as 97% of all the food samples tested. Only two mycotoxins (BEAU and ZEN) and one bacterial metabolite (chloramphenicol) were quantified in the milk samples; the mycotoxin concentrations were low (< 1 μg/kg) whilst the levels of chloramphenicol reached 64 μg/kg (Table 2). The chromatograms of some contaminated food samples are given in Fig. 3.

Overall, *ogi*, family cereal and *Tom bran* samples contained as many as 7, 10 and 12 co-occurring mycotoxins, respectively. The major co-occurring toxins were AFB_1, $FB_1 + FB_2$, CIT, MON and OTA. Infant formula and peanut butter contained mainly AFs and MON which occurred simultaneously, while the milk samples showed no evidence of co-occurrence of any major mycotoxins.

3.3.3. Seasonal variations of mycotoxin levels in complementary foods

The variations of 24 mycotoxins and one bacterial metabolite in the complementary foods based on season of food sampling are given in Fig. 4. The concentrations of AFs in the foods did not significantly vary between sampling seasons, though higher levels were observed in the samples collected during the hot dry season than in those from the warm wet season. Of all the measured mycotoxins, only FA_2, FB_1, FB_3 and total FBs significantly ($p < 0.05$) varied between seasons, with higher levels observed during the warm wet season (Fig. 4).

3.3.4. Mycotoxin concentrations and regulatory levels in complementary foods

In general, the Codex Alimentarius standard providing maximum levels (MLs) for mycotoxins in foods are regarded as internationally accepted. However, as the Codex standard does not include specific MLs for mycotoxins in foods for infants and young children, the MLs established by the EU were used to evaluate the compliance of the analyzed food samples. With regard to T-2 and HT-2 toxins, the non-legally-binding indicative level of the EU was used. This level is not a safety level and above it further investigations should be conducted.

The proportions of complementary food samples exceeding the levels stipulated by the EU legislation for various mycotoxins are presented in Table 3. The levels of AFs, FBs, OTA, T-2 + HT-2 clearly exceeded the EU levels in various complementary food samples. As much as 52% of the *ogi* and 80% of the *Tom bran* samples contained AFB_1 at the levels above the 0.1 μg/kg EU ML for processed baby food, while 39% and 23% of the same food types, respectively, exceeded the

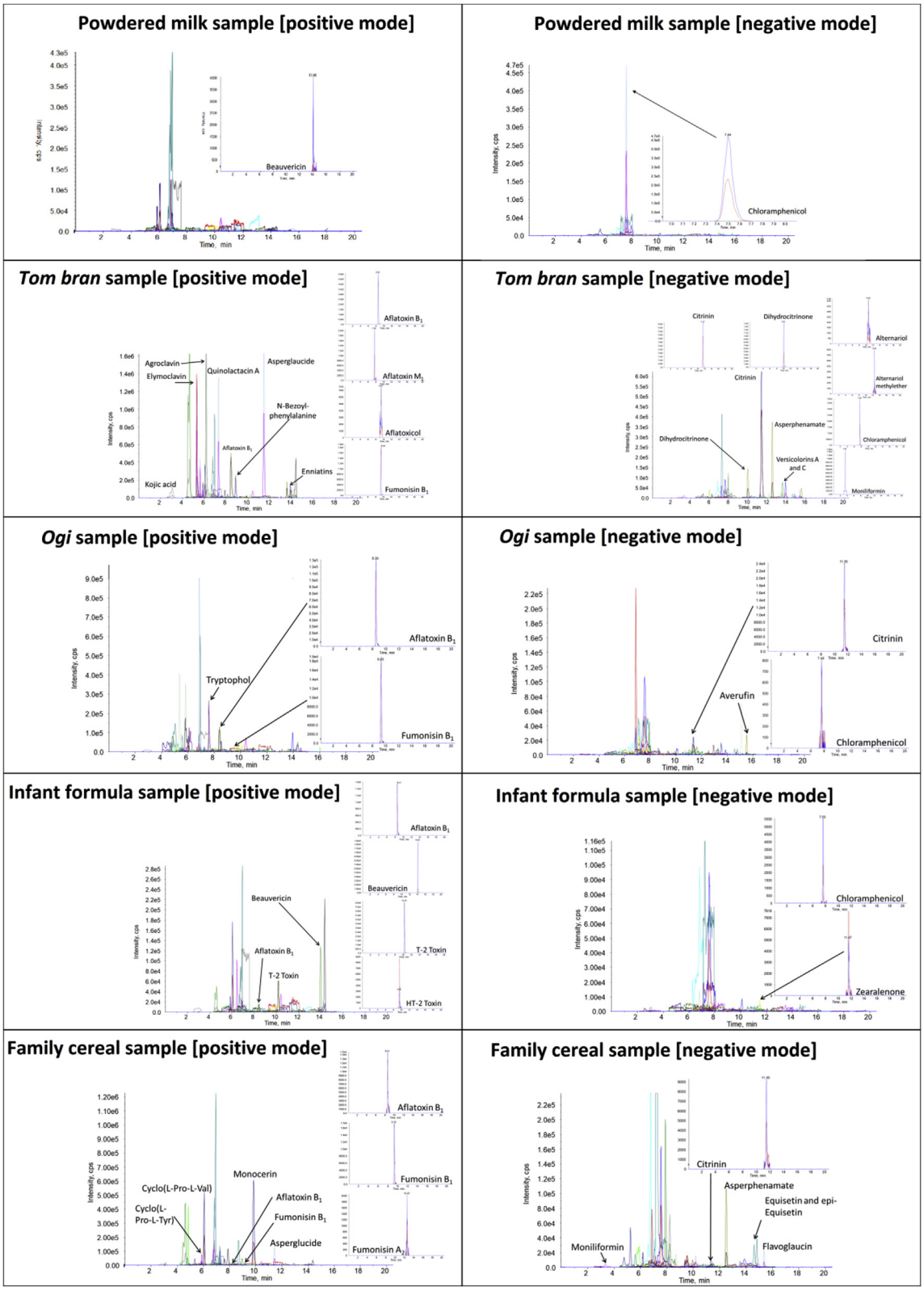

Fig. 3. Chromatograms of mycotoxins and bacterial metabolite found in complementary food samples for infants and young children in Nigeria.

200 μg/kg EU ML for the sum of FB_1 and FB_2 in processed baby food. The 0.5 μg/kg EU ML for OTA in processed baby food was exceeded by 9% of the *ogi* and 27% of the *Tom bran* samples. Only 27% and as much as 80% of family cereal and peanut butter samples, respectively, contained AFB_1 at levels exceeding the 2 μg/kg EU ML for processed cereal-based foods, while total AFs in 19% of family cereal were above the 4 μg/kg EU ML for same category of foods. However, the 0.1 μg/kg EU ML for processed baby food was also applied to family cereal and peanut butter due to the consumption of these two food sets by IYC in Nigeria as observed in this study; consequently, 92% and 80% of the respective food products exceeded this level (Table 3). Overall, the average AFB_1 content in the cereal-based foods exceeded, by at least

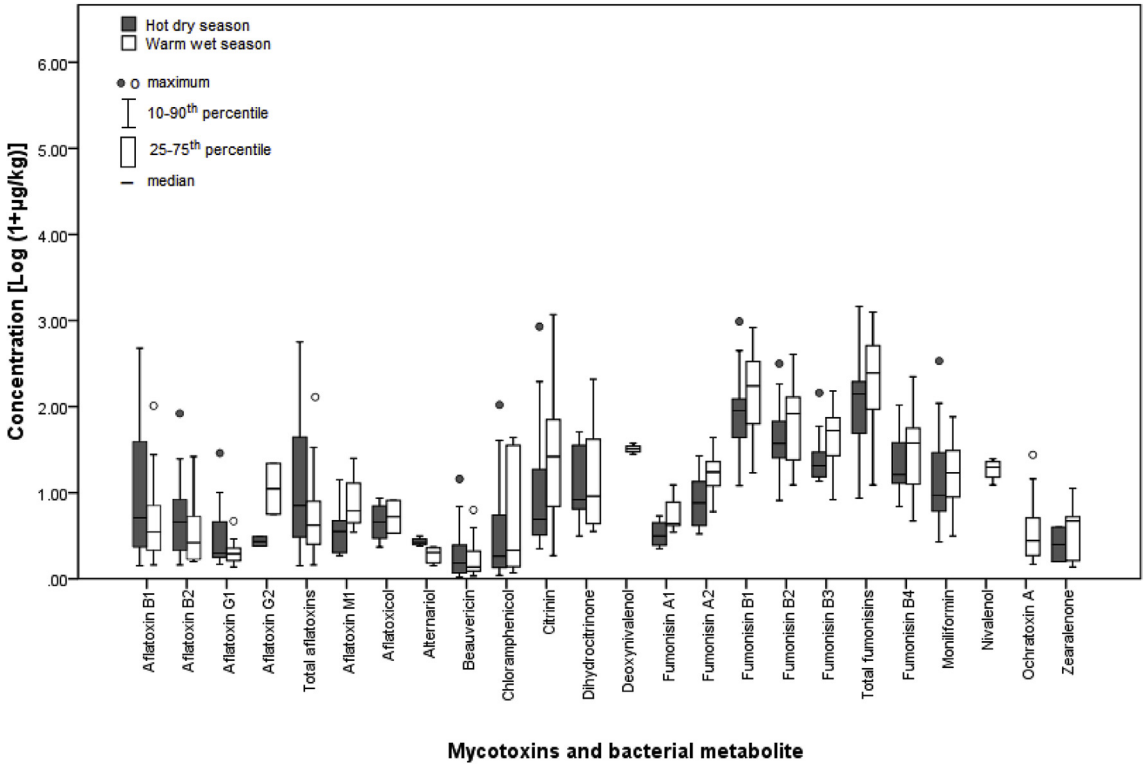

Fig. 4. Variation in mycotoxin and bacterial metabolite levels in complementary foods from two (hot dry and warm wet) climatic seasons in Nigeria. Only differences in levels of FA_2, FB_1, FB_3 and total FBs between seasons were significant ($p < 0.05$).

100 times, the EU ML of 0.1 µg/kg AFB_1 set for processed baby food.

The FB levels in family cereal samples were above the 800 µg/kg EU ML for $FB_1 + FB_2$ in processed cereal-based foods and the applied 200 µg/kg EU ML for $FB_1 + FB_2$ in processed baby food in 9% and 23% of the samples, respectively. The EU ML of 0.1 µg/kg for AFB_1 in processed baby food was also applied to infant formula in this study because the measured infant formula contained a mix of milk and cereals, and also due to the non-detection of AFM_1, which is regulated at 0.025 µg/kg in the EU for infant formula; consequently, 6% of the infant formula samples exceeded the applied limit. The sum of T-2 and HT-2 toxins exceeded, by 9 times, the 15 µg/kg EU indicative level for infant formula in one sample (6%) (Table 3).

3.4. Assessed dietary exposures and health risks from consumption of contaminated complementary foods and identified uncertainties of the assessments

3.4.1. Exposure assessment and risk characterization

The chronic mycotoxin exposures from consumption of complementary foods, reference points and HBGVs used for risk assessments are presented in Table 4. The dietary chronic exposures for IYC consumers were in the ranges of 2.5–51,192 ng/kg bw per day for AFB_1 and 25.7–54,892 ng/kg bw per day for total AFs, resulting in the respective MOEs of 0.003–70 and 0.003–7. For total FBs (sum of FB_1, FB_2, FB_3 and FB_4), the estimated chronic exposures of the IYC was 0.00–138.6 µg/kg bw per day. The maximum exposure was at least 69-fold higher than the group TDI of 2 µg/kg bw per day for the sum of FB_1, FB_2, FB_3 and FB_4. For OTA, the individual exposures ranged from 0 or 0.01 µg/kg bw per week for LB or UB, respectively, to 2.03 µg/kg bw per week. At higher exposure levels, the TWI of 0.1 µg OTA/kg bw was exceeded up

Table 3
Percentage of complementary foods that present mycotoxin levels above the EU maximum acceptable limits.

Categories	Percentage of samples exceeding EU stipulated limits							
	AFB_1	AFM_1	AF total	FUM[d]	OTA	ZEN	T-2 + HT-2	DON
Limits (µg/kg) for processed baby food	0.1	0.05	4[c]	200	0.5	20	15	200
Tom bran (n = 30)	80	47	73.3[c]	23.3	26.7	–	–	–
Ogi (n = 23)	52	4.3	8.7[c]	39.1	8.7	–	–	–
Limits (µg/kg) for processed cereal-based foods	2(0.1)[a]	–	4	800(200)[a]	–	100(20)[a]	–	–
Family cereal (n = 26)	27(92)[a]	–	19.2	8.7(23.3)[a]	–	–	–	–
Peanut butter (n = 5)	80(80)[a]	–	80	–	–	–	–	–
Limits (µg/kg) for infant formula	0.1[b]	0.025	–	–	–	–	–	–
Infant formula (n = 17)	5.9[b]	–	5.9	–	–	–	5.9	–

[a] Limit is applied for this study due to consumption of these foods by IYC in Nigeria.
[b] Limit is applied for this study because infant formula contains cereals.
[c] Aflatoxins limit in Nigeria.
[d] Sum of fumonisins B_1 and B_2.

Table 4
Estimated mycotoxin exposure of children consumers of complementary foods in Nigeria including reference values used for risk assessment and calculated margins of exposure.

	Mycotoxin concentrations and exposure levels				
	AFB_1	Total aflatoxin	Beauvericin	Moniliformin	Citrinin
Mycotoxins[a]	0.12–473.8	0.88–589.8	0.004–69	0.8–3450.2	0.08–1172.7
Exposure[b]	2.5–51,192	25.7–54,892	0–3.14	0.02–156.8	0.002–102
RP[c]	170	170	90	200	0.2
MOE[d]	0–70	0–7	0–30	1–10,000	0–100

	Total fumonisins[f]	OTA LB[g]	OTA UB[h]
Mycotoxins[a]	4.6–1540.4	0–26.4	0.4–26.4
Exposure[b]	0–138.6	0–2.03	0.01–2.03
HBGV[e]	2	0.1	0.1

[a] Range of mycotoxin levels (μg/kg) in foods for infants and young children.
[b] Range of exposure (ng/kg bw per day for aflatoxins; μg/kg bw per week for OTA; μg/kg bw per day for other mycotoxins).
[c] Reference point used for risk assessment; for aflatoxin $BMDL_{10}$ (ng/kg bw per day); for beauvericin a lowest dose of EFSA (2014) (μg/kg bw per day); for moniliformin $BMDL_{05}$ (μg/kg bw per day); for citrinin a level of no concern for nephrotoxicity (μg/kg bw per day).
[d] Range of margin of exposure; rounded to the first significant figure.
[e] Health based guidance value; for fumonisins a tolerable daily intake (TDI) (μg/kg bw per day); for ochratoxin a tolerable weekly intake (TWI) (μg/kg bw per week).
[f] Sum of fumonisins B_1, B_2, B_3 and B_4.
[g] Lower bound values were estimated by assigning zero to values lower than the limit of detection.
[h] Upper bound values were estimated by assigning the value of the limit of detection to values lower than the limit of detection.

to 20 times. The calculated range of MOE for CIT was 0–100 for chronic exposures of 0.002–102 μg/kg bw per day compared to the level of 0.2 μg/kg bw per day at which no concern for nephrotoxicity exists. The estimated range of exposures to MON was 0.02–156.8 μg/kg bw per day and to BEA 0.00–3.14 μg/kg bw per day with the respective MOEs of 1–10,000 and 0–30.

3.4.2. Evaluation of the uncertainties of the exposure and risk assessments for mycotoxins

Evaluation of the uncertainties associated with exposure and risk assessment should accompany the exposure and risk assessment (IPCS, 2009). Therefore, the uncertainties were also evaluated in this study. In the exposure and risk assessment of the Nigerian IYC population, the uncertainties arose mainly from the collection of the daily amounts of consumed food and omission of the food consumption information of other items than the major one per individual. These uncertainties may lead either to an underestimation or overestimation of the exposure. The food consumption data were collected from 110 Nigerian IYC comprising females and males and represent the best available estimates for food consumption habits within this sub-population in Nigeria. The selected food items are the most often fed complementary foods in Nigeria; therefore, the omission of other complementary food items adds only a minor portion to the overall uncertainty of the assessment. The fact that food preparation of *ogi* and *Tom bran* were not considered overestimates the exposure as these food items are diluted before feeding to children. A minor contribution to the overall uncertainty of the exposure assessment originated from the occurrence data generated by the validated LC-MS/MS method.

The application of the middle bound for the occurrence data < LOD contributes to uncertainty of exposure assessment and can either underestimate or overestimate the exposure. The left-censored samples may have contained mycotoxins at higher levels than the middle-bound or they could have been free from mycotoxins. Similarly, the use of LB and UB approach add uncertainty to the exposure estimate; the first underestimating and the latter overestimating the exposure. The application of uncertain reference points for CIT, BEA and MON add uncertainty to their risk assessments, and therefore their risk assessments provide only indications on possible risks. Generally, owing to the available toxicological data, HBGVs and reference points used to characterize risks are typically derived for adults and not for infants and children (WHO, 2006). As the age of the infants in this study was above 16 weeks, it was considered appropriate to use the available HBGVs for adults (EFSA, 2018a). Overall, the exposure and risk assessments for varied mycotoxins were conducted by following the internationally accepted best practices and therefore the assessments are considered to be sufficiently robust.

4. Discussion

This study reports, for the first time, diverse fungal species in processed complementary food samples in Nigeria, as well as the contamination of complementary foods for IYC with as much as 28 mycotoxins and a notable bacterial metabolite. Although, there is no report on fungal contamination of complementary foods in Nigeria, the identified fungal species in this study have been reported in maize and fermented soybean (*moju*) samples from Italy and Korea, respectively (Hong et al., 2015; Logrieco et al., 2014). Furthermore, previous studies from Nigeria that adopted the classical fungal identification method reported several black *Aspergilli* occurring in crops to be *A. niger*, *A. niger* clade or *Aspergillus* section *Nigri* due to the inability to morphologically distinguish members of this group of *Aspergillus* (Adetunji et al., 2014a; Ezekiel et al., 2013, 2016; Oyedele et al., 2017). The black Aspergilli and *A. flavus* have also been reported to occur in household dust/environment worldwide (Jurjevic et al., 2012; Visagie et al., 2014), with few strains linked to respiratory problems in humans (Gautier et al., 2016; Sarubbi et al., 1982). Thus, fungal contaminations reported in this study may have resulted from exposure of the foods to indoor environment given that the food samples were collected from households. This suggests that the food storage practices are not appropriate. Thus, IYC may be faced with health and food poisoning risks by food spoilage microorganisms.

A range of fungal metabolites, including compounds of toxicological importance such as AFs, FBs, CIT, OTA, trichothecenes (DON, T-2 and HT-2 toxins) and emerging mycotoxins (BEA and MON), were found to be prevalent in the complementary foods for IYC in Nigeria as reported in this study. Similar reports on mycotoxin contamination of complementary foods are available in literature for Kenya (Okoth and Ohingo, 2004), Canada (Tam et al., 2006), Turkey (Baydar et al., 2007; Kabak, 2009), Portugal (Alvito et al., 2010), Italy (Juan et al., 2014), and Tanzania (Kamala et al., 2016; Kimanya et al., 2010, 2014).

The mean AFB_1 level quantified in the cereal-based complementary foods (family cereal, *ogi*, *Tom bran* and infant formula containing milk and maize) was more than 40 times the mean value reported by Baydar et al. (2007) for cereal-based food and at least a hundred times higher than the EU regulatory ML for baby food. Such data calls for urgent intervention considering that AFB_1 is a potent human carcinogen inducing hepatocellular carcinoma (IARC, 1993), and reports indicate that the toxin contributes to childhood stunting (Gong et al., 2002, 2016; IARC, 2015). Nevertheless, based on evidence obtained from a recent IARC (2015) evaluation of effects of AFs and FBs on growth of children in low- and middle-income countries, exposure to AFs and FBs contributes to childhood stunting independent of, and together with, other risk factors causing stunting (e.g. undernutrition). In this study, AFB_1 was quantified in almost all the complementary food samples contrary to the Italian infant formula and cereal-based complementary foods (Juan et al., 2014). AFM_1, hydroxylated metabolite of AFB_1 and a group 2B human carcinogen (IARC, 1993), was quantified in *ogi* and *Tom bran* but not in the milk or infant formula, contrary to the previous reports of AFM_1 contamination in milk and milk-based foods for IYC (Alvito et al., 2010; Tsakiris et al., 2013; Er et al., 2014; Kanungo and Bhand, 2014).

It is crucial to mention that the complementary foods analyzed in this study contained AFs above the regulated MLs for these foods in the EU and Nigeria. In fact, as much as 80% of *Tom bran* samples exceeded the AFB_1 level for processed baby food; this is of serious concern giving the widespread consumption of *Tom bran* made from four to five mixed grains, including maize and peanuts. It is therefore imperative for households to review the formulations of grains that are applied in this food type; grains (e.g. soybean, sorghum, and perhaps small underutilized cereals such as fonio millet) that are less prone to AFs may be considered during food formulations. Similarly, family cereal, which had approximately one-fifth of its samples containing AFB_1 and total AFs above the respective 2 and 4 μg/kg limits for processed cereals, recorded as much as 90% violation when the limit was lowered to 0.1 μg/kg based on the "realistic" feeding practices observed at household level. The peculiarity of this food is the fact that mothers and caregivers do not take notice of consumption advice, which excludes the feeding of this product to infants. Reasons for feeding family cereal to IYC by mothers against the given advice were acceptability of product by IYC and perceived nutritional benefits to IYC. Thus, practical solutions for protecting IYC health may include: a) deliberate efforts (e.g. via media campaigns and target group peer trainer seminars) by the health professionals to advice on the health risks due to mycotoxins in foods and to exclude feeding infants with this product; b) setting of appropriate standards or the revision of existing ML for various categories of Nigerian complementary foods. Furthermore, there may be a need for the Nigerian food risk managers to consider applying the 0.1 μg/kg ML for AFB_1 in processed cereal-based foods to infant formula given the inclusion of cereals in infant formula and the outcome of this study.

Taking into consideration the vulnerability of the IYC to mycotoxins, the estimated high dietary exposures to AFs is of great significance to IYC health. According to EFSA (2005), a MOE below 10,000 for a substance that is both genotoxic and carcinogenic, based on the $BMDL_{10}$ from an animal study, is regarded as an indication that the exposure to the substance is of a potential concern for public health and requires risk management actions. In this study, the chronic health risk from the exposure to AFs in complementary foods is considered substantial because a) the calculated MOEs were significantly below 10 000 for all IYC, b) a high proportion of samples were positive for AFs and exceeded the EU MLs – thus, indicating regular high concentrations of AFs in the complementary food commodities, and c) the consumers (IYC) are amongst the most vulnerable population. Therefore, urgent risk management actions to advice consumers to lower the AF concentrations in the foods intended for IYC feeding and to monitor AFs in their foods are required. For chemical compounds which are both genotoxic and carcinogenic, IPCS (2009) recommends that their exposure should be kept as low as reasonably achievable (ALARA). This principle should be applied for AFs in the IYC complementary foods in Nigeria.

FB_1, classified by IARC to be possibly carcinogenic (IARC, 2002), was detected in all the cereal-based complementary foods excluding infant formula containing cereals. Although the FB contents in the foods were high, these levels were lower than those previously reported for FB_1 in complementary food in Tanzania (Kimanya et al., 2010, 2014). It is, however, worrisome that almost a quarter of each of the cereal-based complementary foods contained FBs at levels exceeding the EU regulatory limit for processed baby food. Additionally, the estimated exposures, which exceeded by many folds the group TDI for FBs, indicate a health risk for most of the IYC. The high number of contaminated samples (up to 100%) and in particular, observed high concentrations in the wet season suggest that regular exceedance of HBGV occurs. Consumption of FB-contaminated food has been linked to oesophageal cancer (Rheeder et al., 1992), neural tube defects (Missmer et al., 2006), and growth impairment in children (Kimanya et al., 2010; Shirima et al., 2015; Chen et al., 2018). The lack of regulation for FBs in most food commodities (including foods for IYC) across Africa, and the present observation of a co-occurrence of FBs with AFs in 42% of the 137 analyzed complementary food samples further worsen the scenario. This is because both mycotoxins (FBs and AFs) may interact to impair growth in children (Shirima et al., 2015), although undernutrition and other risk factors are important contributing factors as well (IARC, 2015). Consequently, risk management actions similar to those of AFs would be appropriate. During the wet season when FB concentrations and prevalence can be high, the mothers and caregivers should endeavor to avoid those food items that may increase the risk to dietary exposure to FBs.

The potent nephrotoxic OTA was quantified in the cereal-based complementary food samples (family cereal, *ogi* and *Tom bran*) at average levels that exceeded, by about six times, the EU regulatory limit for processed baby food. The highest concentrations recorded in the samples exceeded also the maximum values reported for infant foods in Turkey and Portugal (Alvito et al., 2010; Baydar et al., 2007) but were below the highest values reported in infant formula in Italy (Meucci et al., 2010). OTA is a class 2B possible human carcinogen (IARC, 1993) with nephrotoxic (Creppy, 2002), genotoxic and mutagenic potentials (Rosa et al., 2004). The risk assessment outcome reveals a potential health concern for many IYC from the chronic exposure due to the exceeded TWI.

CIT, another nephrotoxic (Fuchs and Peraica, 2005; Stefanovic et al., 2006) and possibly a genotoxic carcinogen (Bouslimi et al., 2008; Liu et al., 2003), is reported for the first time to the best of our knowledge in family cereal, *Tom bran* and infant formula. It was, however, recently reported in maize and *ogi* from Nigeria (Okeke et al., 2015, 2018). About 60–80% of the highly consumed cereal-based foods (excluding infant formula) in this study was contaminated with CIT. A higher proportion of these samples containing high levels of CIT leading to no margin between exposure and the level of no-concern for nephrotoxicity suggest a potential health risk for IYC from the exposure to CIT. As substantial uncertainties were associated with the derivation of the reference value (EFSA, 2012), the outcome of this assessment is indicative of possible risk. However, considering that CIT-induced genotoxicity and carcinogenicity could not be excluded at the level of no-concern for nephrotoxicity (EFSA, 2012), similar risk management measures as for AFs are warranted.

The co-occurrence of nephrotoxic OTA and likely genotoxic-carcinogenic CIT, with AFs, FBs, and other major mycotoxins in more than 90% of the food samples analyzed in this study may further increase the health risks in the studied vulnerable population group. Combined effects of multiple food contaminants, such as the combinations reported here, have been mainly elucidated *in vitro* and summarized by Klarić et al. (2013). Background concentrations of OTA/CIT, OTA/CIT/FB_1

and OTA/FB_1 combinations have been reported to exert synergistic effects and combinations of OTA/AFB_1 and OTA/FB_1 additive effects *in vitro* (Creppy et al., 2004; Golli-Bennour et al., 2010; Klarić et al., 2008, 2012; Stoev et al., 2009). However, the relevance of combined effects *in vivo* is still poorly understood. The exposure to a mixture of toxins with interactive effects in the same target organ, in particular with the same mode of action, may enhance incidence of chronic diseases (e.g. renal disease) and cancer (Klarić et al., 2013; EFSA, 2018b).

Other toxicologically important mycotoxins that were found to occur at high frequencies in the complementary food samples include alternariol, BEA, MON, T-2 and HT-2 toxins. Alternariol is a possible genotoxic carcinogen (Lee et al., 2015; Ostry, 2009; Rychlik et al., 2016) that was quantified in all the cereal-based foods including infant formula. Similar findings have been reported by Rychlik et al. (2016). There is no previous reports of BEA and MON in baby foods in Nigeria and in milk globally, although BEA was found to contaminate infant cereals in other countries (Blessa et al., 2012; Juan et al., 2013). However, a recent report found BEA to be the dominant mycotoxin in human breast milk in Ogun state, Nigeria (Braun et al., 2018). The low MOEs calculated for BEA and its high occurrence in the regularly consumed cereal-based complementary foods indicate a potential health concern for IYC. The exposures to MON suggests a potential health concern particularly for regular family cereal consumers. However, like EFSA, no firm conclusions could be made of the risks posed by the BEA and MON exposures owing to the uncertainties associated with the derived reference points.

The dietary exposure to most of the mycotoxins appears to be regular. This increases the risks of adverse health effects when HBGV or other reference points are repeatedly exceeded. Even if the contribution of the exposure from the separate food items to a total daily exposure was not estimated, it is reasonable to conclude that the exposure from peanut butter contributed less to the daily mycotoxin exposure because it is consumed as a spread. In contrast, the major contributing food item to the mycotoxin exposure was *Tom bran* due to the highest concentrations observed and given its frequent consumption. It is noteworthy that other foods not considered in this study but fed to IYC may also contribute to the total mycotoxin exposure.

The trichothecenes, immune- and haematotoxic T-2 and HT-2 toxins (EFSA, 2017), that were quantified in the infant formula samples at levels three times higher than the EU indicative level, is noteworthy because these mycotoxins have not been previously reported in processed foods in Nigeria. Although the EU indicative level is not a safety level, this finding calls for further investigations. The presence of the bacterial metabolite, chloramphenicol with antibiotic properties, in the complementary foods (including milk and infant formula) demands attention as it is critical to avoid unwanted antibiotic exposure in this vulnerable population.

Overall, the co-occurrence of major mycotoxins in the complementary food samples is similar to previous reports on co-occurrence of these mycotoxins in individual grains used in production of complementary foods (Geary et al., 2016; Hove et al., 2016; Kamala et al., 2015, 2016; Kimanya et al., 2008, 2014; Smith et al., 2016). Higher co-occurrence in *Tom bran* may have been facilitated by the different grains used in the formulation of this complementary food. The recorded low-to-moderate concentrations of the emerging mycotoxins and other metabolites, whose toxicities have not been well documented in literature, should not be overlooked due to the recent reports of synergistic interactions of background concentrations of emerging toxins with major toxins (Vejdovszky et al., 2016, 2017a; 2017b).

Seasonal variations in the levels of some mycotoxins were observed in the complementary foods. The variations are arguably linked to the quantities of mycotoxins in the grains/raw materials used in the production of these complementary foods as opposed to post-production contamination. Many mycotoxins are known to be produced in larger quantities at higher temperatures and due to increased rainfall frequency (Bandyopadhyay et al., 2007; Marin et al., 1995; Ono et al., 1999; Visconti, 1996; Viquez et al., 1996).

5. Conclusion and recommendations

This study has shown that fungi and mycotoxins in addition to bacterial metabolites (e.g. antibiotics) are common contaminants of complementary foods produced in Nigeria. The presence of several major and emerging mycotoxins in the baby food samples highlight the need to prioritize routine surveillance and monitoring of these foods by the regulatory authorities. The high chronic exposure estimates for several different mycotoxins reveal an enormous health risk for the general IYC population fed with complementary foods in Nigeria. Presently, regulations for mycotoxins in baby foods in Nigeria are clearly inadequate as demonstrated by the adoption of a limit of 4 μg/kg for AFs in the foods compared to the EU limit of 0.1 μg/kg. Furthermore, other major mycotoxins reported in this study are not regulated in baby foods in Nigeria. Therefore, a revision of the AF regulation and considerations for the regulations of FBs and OTA is urgently required in view of the higher vulnerability of infants than other age groups to the adverse effects of mycotoxins. For CIT, the ALARA principle should be adopted for foods consumed by IYC in addition to routine monitoring and risk assessment of all mycotoxins including T-2 and HT-2 toxins. Furthermore, awareness/educational interventions are required to enhance caregiver adherence to consumption advice for specific foods while adopting the grain replacement technique during formulation of the foods.

Conflicts of interest

The authors declare they have no competing financial interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.fct.2018.08.025.

Transparency document

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